

Purity Analysis of SRM Cholesterol, Estradiol, Progesterone, and Testosterone Steroids by Proton NMR

Final installation of our 600 MHz NMR instrument was completed in the fall of 2005. Almost immediately the usefulness of our new NMR capabilities was tested by requests to aid in the purity analyses of several SRM steroids.

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Estradiol, progesterone, and testosterone samples, selected as primary standards for the certification of SRM 971 - Steroids in Human Serum, were each quantitatively analyzed by NMR.

Cholesterol. NMR analyses were performed to compare the SRM 911c and 911b cholesterol and to verify the presence of, and identify, a 911c impurity with a mass of 384 (=cholesterol mass-2) that had been observed by LC MS analysis of the 911c material. Based on the LC-MS results, the major impurity in the 911c material was thought to be 5,7-cholestadiene-3 β -ol, but NMR easily ruled this out. The NMR spectra however suggested the presence of 5,24-cholestadiene-3 β -ol, and its presence was confirmed by spectral comparison with a commercially available sample. Identification of this impurity allowed accurate quantification of the impurity by LC techniques. In addition to the major impurity three minor impurities were also present in 911c. Two of these minor impurities were tentatively identified as 5,25-cholestadiene-3 β -ol and cholest-7-en-3 β -ol, but standards were not available for confirmation. Although the NMR experiments in these analyses were performed to provide screening results, rather than optimal quantification, the quantitative results: 0.57 mole % 5,24-cholestadiene-3 β -ol and 0.08 mole % 5,25-cholestadiene-3 β -ol were in agreement with LC results. The other two minor impurities were present at 0.06 mole % and 0.02 mole %.

Estradiol. NMR spectra showed one major impurity in the estradiol sample. The impurity, present at 0.38 mole %, was initially tentatively identified as estrone. NMR

analysis of an estrone sample confirmed the estrone identification. The NMR spectrum of estradiol is shown below. The inset is an expansion of the spectrum showing the doublet of doublets from the H-18 estrone impurity. In addition to the major impurity two minor impurities were present at 0.025 mole % and 0.0067 mole %. These minor impurities were not identified. Due to the severe spectral overlap of closely related structures very limited spectral data for the minor components are often present, making identification difficult, if not impossible.

Progesterone. One major impurity was present in the NMR spectra of the progesterone sample. This impurity, present at 0.86 mole %, is tentatively identified as 4-pregnen-3-ol-20-one. A commercial sample is backordered so the identity has yet to be confirmed. The NMR spectra suggest the presence of several other minor impurities, though the resolved data is very limited so the data is not definitive. Quantification of these impurities range from 0.3 mole % to 0.05 mole %.

Testosterone. The major impurity in testosterone was identified and confirmed by NMR spectroscopy as epitestosterone (17 α -hydroxyandrost-4-en-3-one). The epitestosterone is present at approximately 0.29 mole %. NMR spectra show the presence of two additional unidentified minor impurities at 0.05 mole % and 0.04 mole %.

NIST is applying new NMR technology to improve the quality and reliability of its measurements NIST's steroid Standard Reference Materials.

